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FURTHER STUDIES ON HETEROCHROMOSOMES IN MOSQUITOES.

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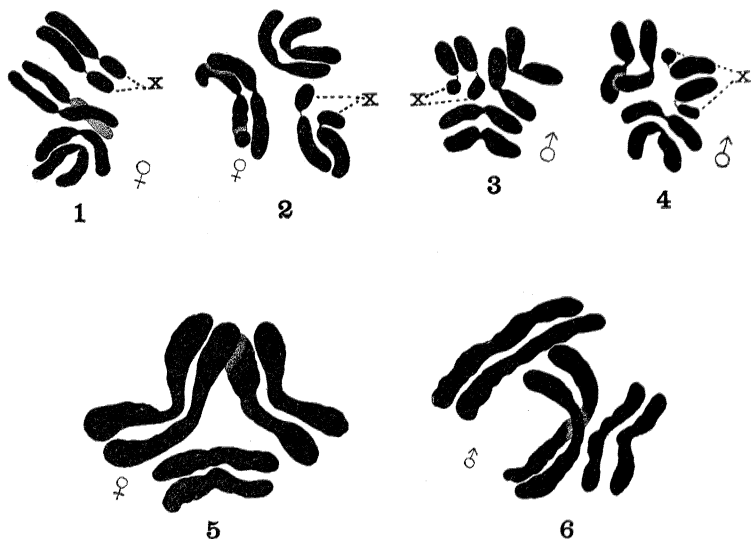
In my study on the chromosomes in the germ cells of *Culex*, published in March, 1910 ('10), there were three figures (11, 20 and 21) which differed from all others for *Culex*, in showing an unequal pair of chromosomes in the spermatogonia (Fig. 11), and a condensed pair in the growth stage of the spermatocytes (Figs. 20 and 21). At the time when the proof of that paper was corrected, it was suspected that the three figures referred to belonged to another species, probably *Anopheles punctipennis*, a few larvæ of *Anopheles* having, it was supposed, been collected in the net with *Culex*, and having escaped detection in both larva and pupa stage.

In October of this year (1910) an abundant supply of *Anopheles* larvæ was secured and a satisfactory study of both male and female germ cells made, showing without question that in the male *Anopheles punctipennis* there is present an unequal pair of heterochromosomes, attached to one of the equal pairs, but otherwise exactly comparable to the unequal pair described by the author for nine species of Muscidae ('08). A careful reëxamination of the germ cells of *Culex pipiens*, and a study of *Culex tarsalis* and *Theobaldia incidens* in California during the summer, revealed no such heterochromosomes in those species. A description of the chromosomes in the germ cells of *Anopheles punctipennis* and *Theobaldia incidens* will be given in the following pages.

Methods.—The germ glands of both *Anopheles* and *Theobaldia* were studied both in acetocarmine preparations and in sections of material fixed in Flemming and stained with iron-hæmatoxylin or thionin. The latter stain gave very satisfactory results with this material, and saved much time in preparing the sections for study.

Anopheles punctipennis.

Very clear oögonial plates were found in several ovaries, and two of these are shown in Figs. 1 and 2. There are two equal



FIGS. 1 and 2. *Anopheles punctipennis*, chromosomes of the oögonia. Mag. 2,000.

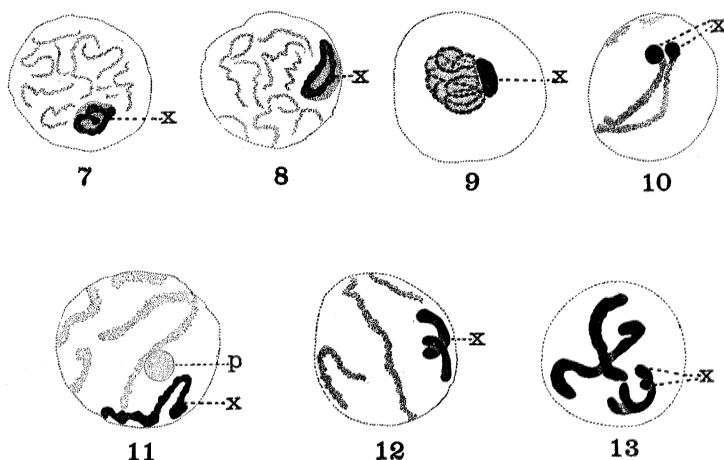
FIGS. 3 and 4. *Anopheles punctipennis*, chromosomes of the spermatogonia. Same mag.

FIG. 5. *Culex pipiens*, chromosomes of an oögonium. Same mag.

FIG. 6. *Culex pipiens*, chromosomes of a spermatogonium. Same mag.

pairs of chromosomes very similar to those found in other Diptera, and a third pair which is clearly made up of a longer and a shorter pair. Corresponding figures of spermatogonial plates are shown in Figs. 3 and 4. Here the composite pair consists of a longer equal pair, as in the oögonia, united with a smaller unequal pair, the larger member of which is of the same relative size as the members of the corresponding small pair in the oögonial plates. All of the spermatogonial plates in this *Anopheles* material were of this type. In *Culex*, on the other hand, no such inequality or composite condition of one pair of chromosomes could be detected. Figs. 5 (♀) and 6 (♂) are new figures of the oögonial and spermatogonial chromosomes of *Culex pipiens*, and those of *Culex tarsalis* are of the same general character.

In resting spermatogonia of *Anopheles* (Figs. 7 and 8) a condensed mass of chromatin varying in form is found associated with a large plasmosome near the nuclear membrane. In *Culex* there is nothing of the kind—only a large central plasmosome surrounded by an even spireme. In *Anopheles* there is a very conspicuous contraction or synizesis stage (Fig. 9) in which the



FIGS. 7 and 8. *Anopheles punctipennis*, resting spermatogonia. X = the heterochromosome.

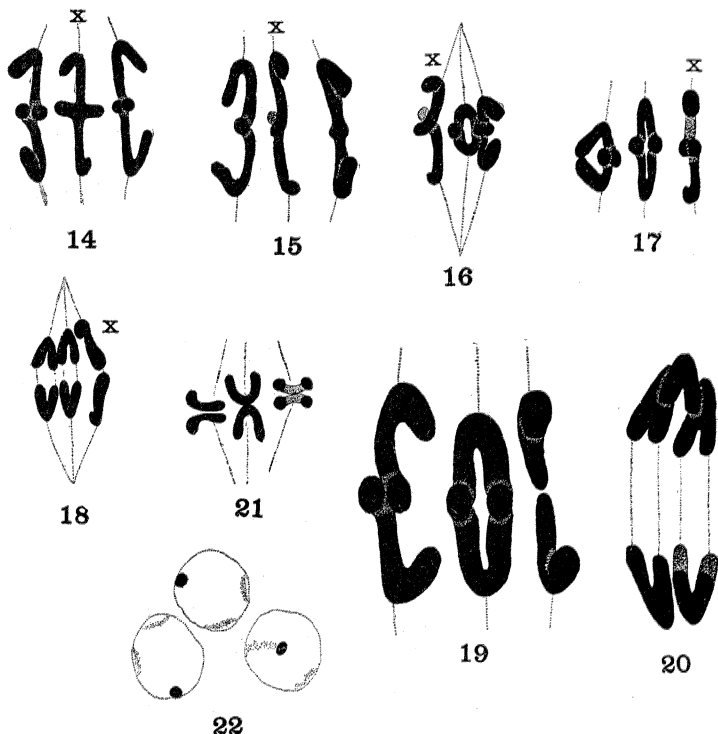
FIG. 9. Synizesis stage.

FIGS. 10-12. Growth stages.

FIG. 13. Prophase.

heterochromosome appears as a deeply staining mass of chromatin resting against a ball of paler chromatin threads. The corresponding stage in *Culex* ('10, Figs. 12 and 13) showed a ball of chromatin thread wound about a plasmosome. In the later growth stages of the spermatocytes of *Anopheles*, the heterochromosome pair assumes a great diversity of forms. Occasionally, especially in early stages, the unequal ends of the pair only are fully condensed as shown in Fig. 10, which is a tangential section of the nucleus. Fig. 11 shows a late spireme stage with heterochromosome (x), plasmosome (p) and parts of a pale spireme. Fig. 12 is a late growth stage taken from a cyst where the cells were mostly in metaphase of the first spermatocyte mitosis; here the heterochromosome pair shows nothing of its composite

nature, and the members of the pair are in about the position which they assume in the spindle. Throughout the growth stage the composite pair of chromosomes is found, more or less condensed, but always distinguishable from the remainder of the chromatin—against the nuclear membrane, twisted or coiled or



FIGS. 14-17. *Anopheles punctipennis*, first spermatocyte metaphase. X = the unequal pair.

FIG. 18. Anaphase.

FIGS. 19 and 20. *Culex pipiens*, first spermatocyte metaphase and anaphase.

FIG. 21. *Anopheles punctipennis*, second spermatocyte metaphase.

FIG. 22. Spermatid nuclei. All figures same magnification—2,000.

knotted into such shapes that it is usually impossible to distinguish the equal and unequal pairs of which it is composed.

Prophase stages are evidently much briefer than in *Culex*, for it was difficult to find any clear figures—Fig. 13 shows the composite heterochromosome pair (x) and the other two chromosomes not yet separated into their parallel components.

The character of the chromosome pairs as they appear in the first maturation mitosis is well shown in Figs. 14-17, where the chromosomes are represented in one plane for the sake of clearness. In each figure x is the composite pair. The spindle fibers appear to be attached at the meeting point of the smaller and larger components, the smaller, unequal pair forming the outer ends of the figure and appearing as a conspicuous hook at one end and a rounded knob at the other. No distinct separation of the members of the composite pair, such as is always seen in the spermatogonia (Figs. 3 and 4) was ever observed in the spermatocyte metaphase. The other two chromosomes frequently assume the form of rings in the early metaphase (Figs. 16 and 17) and in the anaphase (Fig. 18) they separate as V's with equal arms, while the heterochromosomes form a pair of hooks with equal long arms and unequal short arms (Fig. 18).

Fig. 19 shows the chromosomes of a first spermatocyte metaphase of *Culex pipiens* and Fig. 20 an anaphase. The chromosomes correspond very closely in form and relative size to those of *Anopheles* with the exception that all three pairs are equal. Both cells and chromosomes are much larger in *Culex* than in *Anopheles*.

In the second spermatocytes the chromosomes are already divided when they come into the spindle and are usually so tangled as to defy any attempt to distinguish the two classes or make clear drawings. In Fig. 21 we have an unusually clear metaphase. An aggregation of chromatin, apparently the heterochromosome, is found in the spermatids (Fig. 22), lying against the nuclear membrane, and in some cases the dimorphism can be distinguished.

Theobaldia incidens.

Theobaldia incidens is one of the most common mosquitoes in the Santa Clara Valley, California. The larvæ were found in abundance in a bucket of water which had been allowed to stand partly covered for two or three weeks. A few bread crumbs thrown into the glass jars in which the larvæ were kept under observation, enabled them to grow rapidly and transform into fine large pupæ whose germ glands yielded all desired stages, from dividing oögonia and spermatogonia to ripe spermatozoa.

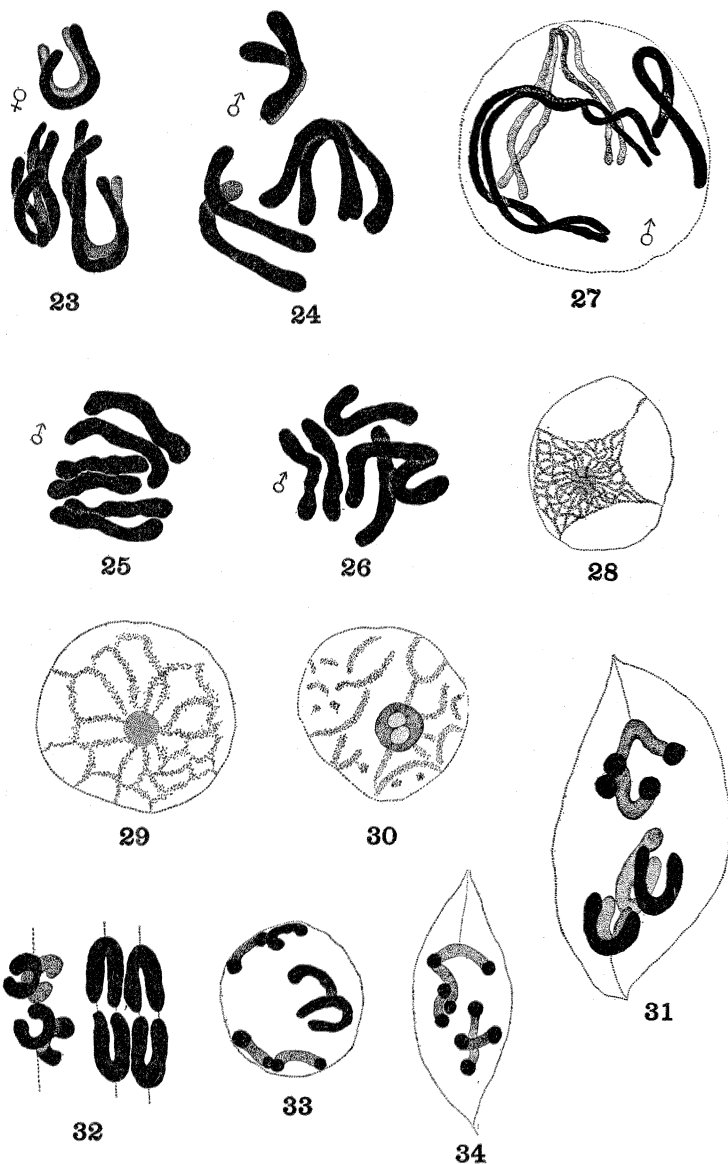


FIG. 23. *Theobaldia incidens*, oögonial prophase.

FIGS. 24-26. Chromosomes of the spermatogonia.

FIG. 27. Spermatogonial prophase.

FIG. 28. Synizesis stage.

FIGS. 29 and 30. First spermatocyte growth stages.

FIGS. 31 and 32. First spermatocyte prophase and metaphase.

FIGS. 33 and 34. Second spermatocyte prophase.

During the summer only acetocarmine preparations were used and the material resembled *Culex* so closely that only a few figures were made. Other figures were taken from sections of ovaries and testes fixed in Flemming and stained with thionin. An oögonial prophase is shown in Fig. 23, and the chromosomes from three spermatogonia in Figs. 24, 25 and 26. Fig. 27 is the nucleus of a spermatogonium in prophase. In no one of these figures is there any indication of inequality in the members of any pair of chromosomes. Neither in resting spermatogonia nor in the growth stages of the spermatocytes could anything resembling the heterochromosomes of *Anopheles* be found. In all of these stages a central plasmosome is surrounded by a spireme as in *Culex*. Figs. 28 to 30 show a contraction or synizesis stage, and two later growth stages. As in *Culex* the plasmosome stains a deeper blue in late growth stages than in the spermatogonia and synizesis stages. Fig. 31 is a characteristic prophase of the first spermatocyte mitosis. In the metaphase two of the chromosome pairs nearly always appear in the form of rings and separate as V's; the third pair is usually separated and its univalent components already divided longitudinally, when the rings are just dividing (Fig. 32). The second spermatocyte prophases (Figs. 33 and 34), metaphases and anaphases resemble closely those of *Culex*.

The testes of *Theobaldia* differed from those of *Culex* in having much larger spermatogonia and in lacking a prolonged prophase of the first spermatocyte mitosis. The first maturation division also shows characteristic differences in the behavior of the chromosome pairs in metakinesis (compare Figs. 19 and 32). Otherwise both ovaries and testes showed similar conditions in the two species, *Culex pipiens* and *Theobaldia incidens*; i. e., three equal pairs of chromosomes, no one of which behaves in any respect like the heterochromosomes of other insects, including *Anopheles punctipennis*.

Anopheles sp.?

On March 30 and again on May 31, a few pupæ were obtained from the same pond where I obtained my material in 1909. All of the males in these collections had six chromosomes in the spermatocytes and a distinct heterochromosome in the growth

stages. The pupæ were thought to be *Anopheles* but no larvæ or adults were secured for identification. It was supposed that the few specimens obtained must have wintered over, probably in

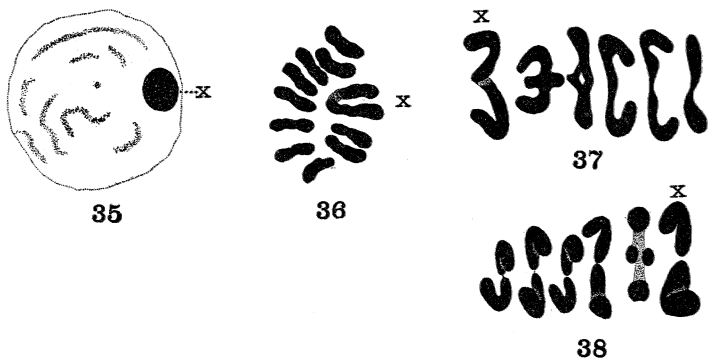


FIG. 35. *Anopheles* sp.? First spermatocyte growth stage. X = the heterochromosome.

FIG. 36. First spermatocyte prophase.

FIGS. 37 and 38. Chromosomes of the first spermatocyte metaphase. Mag. 2,000 in all figures.

the larval stage. The net was used in the pond many times during March, April and May, but no mosquito larvæ or pupæ secured except on the two dates given above.

Fig. 35 shows the heterochromosome in the nucleus of a growth stage, Fig. 36 a prophase of the first maturation mitosis, and Figs. 37 and 38 the six chromosomes from two first spermatocyte spindles. The largest pair is slightly unequal but not composite as in *Anopheles punctipennis*.

DISCUSSION.

As we have already seen, the various species of Culicidæ, thus far studied, present peculiarities not hitherto encountered in the study of heterochromosomes in the various groups of insects.

In *Anisobola maritima* (Randolph, '08) and several species of Lepidoptera (Stevens, '06; Dederer, '07; Cook, '10) there is an equal pair of heterochromosomes distinguishable as such by their condensed condition in the growth stages of the first spermatocytes. In *Drosophila ampelophila* the unequal pair of heterochromosomes of the maturation mitoses is not condensed

during the growth stages (Stevens, '08). In *Metapodius terminalis* (Wilson, '09) we find either an unpaired heterochromosome or an unequal pair of idiochromosomes, and a varying number of supernumeraries within the same species. In no case before have undoubted heterochromosomes, following the usual rule as to sex distribution, been found in one or more genera or species and entirely absent in nearly related species of the same family. In *Anopheles punctipennis* the heterochromosomes can be traced from the youngest spermatogonia observed, through the two generations of spermatocytes to the spermatids and even to a stage in which the spermatids are transforming into spermatozoa. The unequal pair in the male is represented by an equal pair in the female, and as in other similar cases, it is evident that an egg fertilized by a spermatozoön containing the smaller heterochromosome must produce a male, while an egg fertilized by a sperm containing the larger heterochromosome will develop into a female.

Now in *Culex* and *Theobaldia* all of the chromosomes behave alike so far as condensation is concerned, and there is neither an unpaired heterochromosome nor an unequal pair to be detected in any stage. In other words there is no visible differentiation of one pair of chromosomes which can be associated with the determination of sex. There seems to be absolutely no reason for supposing that the mechanism by which sex is determined is not identical in these three species of mosquitoes; and the inevitable conclusion would seem to be either that the heterochromosomes of the male are not a necessary factor in sex determination, or that the sex-determining chromosomes are not necessarily differentiated as heterochromosomes.

I have kept no record of the sex of the pupæ dissected, but should say that the numbers of males and females were about equal for all of the species studied. In a large collection of *Theobaldia* larvæ which, judging by their size, might have all come from one laying, most of the males pupated a day or two sooner than the females. All of the pupæ were taken out and dissected each day. The first day nearly all were males and the last day nearly all females. An average for the four or five days, I feel sure, must have given nearly equal numbers.

Wilson's recent hypothesis ('09) that in some way two *X* chromosomes determine the female sex, and one *X* chromosome or an *X* and a *Y* chromosome determine the male sex would apply perfectly to *Anopheles*.

Egg $X + sp.$ $X = \text{♀}$,

Egg $X + sp.$ $Y = \text{♂}$.

But what shall we say of *Culex* and *Theobaldia* where there is no differentiation of *X* and *Y* chromosomes? In forms which have an equal pair of heterochromosomes, showing the same general characteristics as the unpaired heterochromosomes and the idiochromosomes, one naturally infers that the members of the equal pair in the male have *X* and *Y* values respectively, but shall we go further and argue that likewise in *Culex* and *Theobaldia* the mature eggs all have an *X* chromosome and the spermatozoa are dimorphic, one half having an *X* chromosome and the other half a *Y* chromosome, although nothing of the kind can be traced? If we make this assumption, then *Culex* and *Theobaldia* may stand at one end of a series, as examples of absence of heterochromosomes associated with sex, while forms with an unpaired heterochromosome come at the other end of the series, and all the varieties of paired heterochromosomes between, the mechanism of sex determination being the same in all, but evident to the eye only when differential heterochromosomes are present.

The case of the aphids and phylloxerans has been the strongest argument for the hypothesis that two *X* chromosomes give a female and one *X* or *XY* a male, since the rejection of one *X* chromosome from a parthenogenetic egg is followed by the development of a male, but in *Culex* and *Theobaldia* it is evident that sex determination is not dependent on the presence of *X* and *Y* chromosomes, although in *Anopheles* these chromosomes are at least closely correlated with sex-determination.

At present, the all-important questions seem to me to be: What is the meaning of the differentiation of heterochromosomes in one form and not in others closely related? What has been the history of such differentiation where we have an unpaired heterochromosome or an unequal pair of heterochromosomes?

In *Metapodius* Wilson ('09) traces the origin of an unpaired heterochromosome and also of the supernumeraries to an irregular mitosis in which both idiochromosomes go to one daughter cell. In the *Diabroticas* the supernumeraries probably owe their origin to an irregular division of the unpaired heterochromosome.

But in no case are we able to say when or how or why certain spermatogonial chromosomes became specially differentiated as heterochromosomes.

We have associated the *X* and *Y* chromosomes of the male with sex-determination, but possibly they have some other meaning and are only so correlated with the sex-determining mechanism that they have the same distribution as the sex characters. The case of the mosquitoes certainly indicates that we must study the heterochromosomes, apart from the idea of sex-determination, more intensively, in order to determine if possible why we have such chromosomes differentiated in some forms and not in others.

In such cases as *Anopheles* the sex characters may be supposed to be located in the unequal heterochromosomes or in the larger equal pair with which they are connected. This may also be true of the composite heterochromosomes of *Drosophila ampelophila* and *Hesperotettix* (McClung, '05). In the ordinary cases of an unpaired heterochromosome or an unequal pair, we may suppose the sex characters to be located in the heterochromosomes, but to be independent of the special differentiation, which may be entirely absent as in *Culex* and *Theobaldia*.

If we apply Morgan's new sex formula—

$$FmFm = \text{♀}, \quad Fmf m = \text{♂},$$

to the cases under discussion, *m* may be located in any equal pair but *F* must be either in the heterochromosomes or in chromosomes correlated with them. Sex-limited inheritance in the male (Morgan, '10) would then seem to be possible only where unpaired or unequally paired heterochromosomes are present, and not in such cases as, *Anisoloba*, the Lepidoptera, *Culex* and *Theobaldia*.

It occurs to me as possible that heterochromosome differentiation may be directly related to sex-limited inheritance of certain

characters. If so we should expect to find an unpaired or an unequally paired heterochromosome in the female of *Abraxas* where there is sex-limited inheritance in the female, and also to find sex-limited inheritance of some character or characters in the female of those echinoderms in which Baltzer ('09) and Heffner ('10) have shown that an unequal pair of chromosomes is present in the female. Apparently we have reached a point where the experimental breeder and the cytologist *must* work together in order to solve the problems of sex-determination, sex-limited inheritance and heterochromosome differentiation.

BRYN MAWR COLLEGE,
December 20, 1910.

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